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IN THE MATTER OF
US Patent Application No. 09/380,327
by ROBERTSON et al.

Declaration under U.S.C.§ Rule 132

STATUTORY DECLARATION

I, David Alexander Clark of 444 Smith Ave., Burlington in the Province of Ontario, Canada, do solemnly and sincerely declare as follows:

1. I am the same David Alexander Clark who made a statutory declaration dated 16 December 2002 in respect of this matter.

2. I have read and understood the second office action dated 11 March 2003 issued in respect of this application. I have also read and understood the claims as presently on file, and as now proposed to be amended.

3. I note that as a result of these amendments the claims now require that

a) the antigen to which the prospective mother is exposed is either a sperm antigen, or an MHC Class I antigen of the prospective father which is present on leukocytes or in the seminal plasma of the prospective father,

b) the TGFβ is selected from the group consisting of $TGF\beta_1$, $TGF\beta_2$, $TGF\beta_3$ and activin, and

c) the method is directed to treating an infertility condition.

d) the method is directed to inducing immune tolerance.

4. The Examiner has stated at page 14 of the office action

> "In response to Applicant's argument that the novel $TGF\text{-}\beta_2$ in Clark et al is different from the conventional isoform of TGFβ, and one would not have expected that administering a conventional TGFβ isoform would prove beneficial in preventing infertility, the claims are not drawn to preventing infertility. Further, claims 75 to 76 recite TGFβ is modified comprises (sic) substitution, deletion, or addition mutants or peptide fragments of TGFβ which are clearly non-conventional TGFβ."

I understand that, while the applicants do not concede the correctness of the Examiner's objection on this point, claims 75 to 77 are to be cancelled without prejudice, and that the applicants are reserving the right to pursue these claims via a divisional application.

5. As a result of this amendment, the claims no longer encompass substitution, deletion, or addition mutants or peptide fragments of TGFβ, ie. do not encompass non-conventional TGFβ.

6. Furthermore, the Examiner has stated at page 12 of the office action:

> "Clark *et al* teach bioactive TGFβ is known to suppress the generation of cytotoxic cells in vitro and has immunosuppressive activity that leads to induction of tolerance in vivo during the first trimester pregnancy in humans".

I consider that the Examiner is completely incorrect in her interpretation of the Clark *et al.* reference, of which I am first author.

7. As discussed in Clause 26 of my previous declaration, the Clark *et al.* reference discloses the up-regulation of release *in vitro* of *non-conventional* TGFβ from $CD56^+$ cells obtained from decidua of human first trimester pregnancy. The non-conventional TGFβ was assayed *in vitro*

using mouse cytotoxic T lymphocyte (CTL) generation. This assay was chosen as a means to confirm that the non-conventional TGFβ exhibits one of the known biological activities of TGFβs.

8. However, the results from this assay alone do not indicate or suggest the biological function of *non-conventional* TGFβ ***in vivo*** and, therefore, the Clark *et al.* reference does not teach that the *non-conventional* TGFβ has immunosuppressive activity *in vivo*. In fact, the significance of the presence of the *non-conventional* TGFβ during the first trimester of pregnancy as shown by the Clark *et al.* reference was unknown; this is not surprising, as TGFβs were known to exhibit many biological functions, including cell division, cell growth inhibition, angiogenesis, cell migration, cell differentiation and immunosuppression. It was reasonable to assume that, at the time the present application was filed, the role of *non-conventional* TGFβ during the first trimester of pregnancy, as shown by the Clark *et al.* reference, could be to fulfil any one of the above–listed biological functions.

9. Infertility in humans and in mice was not ascribable in 1997 to lack of "immunological tolerance" to paternal antigen wherein maternal cytotoxic T lymphocytes were prevented from recognizing and rejecting allogeneic embryos. It was the understanding of a person skilled in the art in 1997 that embryo failure was related to natural killer (NK) cells, which lack T cell receptors for antigens such as paternal antigens, and are therefore not specific for any antigen. In 1997, non-antigen-specific NK cells were not considered to be part of the antigen-specific immune system that includes T and B lymphocytes. "Immunological tolerance" refers to an alteration of *antigen-specific* immune function, not non-specific cells such as NK cells; the CTL assay used by Clark et al was merely a convenient *in vitro* bioassay for immunosuppressive molecules such as TGFβ, which can suppress antigen-specific T cell responses *in vitro*.

10. The Examiner's interpretation that Clark et al teaches that *non-conventional* TGFβ has immunosuppressive activity *in vivo*, which leads to induction of immune tolerance *in vivo* during the first trimester pregnancy in humans, is incorrect. In fact, even if Clark *et al* did teach that TGFβ has immunosuppressive activity *in vivo*, this does not equate to or even foreshadow immune tolerance. Suppression of antigen-non-specific NK cells related to spontaneous abortion is not "immune tolerance", which is generally understood to be defined as a lack or deficit of *antigen-specific* immune activity. This contrasts with induction of tolerance as it applies to Chaouat et al,

because in this murine model, an active response to paternal antigen(s) was required to suppress NK cell activity. This is distinct from the "immune tolerance" required to accommodate pregnancy as defined in the applicant's specification.

11. The Examiner has also stated that my declaration states that "one would therefore not have expected that administering a conventional TGFβ isoform would prove beneficial in preventing infertility", whereas the claims are not drawn to *preventing* infertility. I note that claim 1 now defines "a method of treating an infertility condition in a mammalian prospective mother...".

12. I consider that the comments in clause 26 of my previous declaration are equally applicable to a method of treating an infertility condition in a mammalian prospective mother, as this is to be understood in the context of the subject specification.

13. I have reviewed the Examiner's comments regarding the remarks in my earlier declaration about the references by Feinberg and Chaouat, and see no reason to change my previous opinion. I consider that the Examiner has not fully appreciated the implications of my previous comments.

14. The Examiner has stated on page 12 of the Office Action that it would have been obvious to combine the teaching of Feinberg *et al.* and Clark *et al.* with the method of immunizing the female with paternal leukocyte antigen as taught by Chaouat *et al.* However, I consider that the Examiner is completely incorrect in her interpretation of the teachings of Chaouat in the light of the prior art. I therefore do not consider that it would be obvious to combine the teaching of Chaouat *et al.* and Clark and Feinberg and arrive at the applicant's invention.

15. Chaouat discloses immunizing a female *CBA/J* mouse with *BALB/c* leukocytes from spleen, which carried the paternal DBA/2 MHC class I antigen. Immunity elicited by administering paternal strain DBA/2 spleen leukocytes was not protective. It was not necessary to use spleen cells from male BALB/c mice; BALB/c spleen cells from female mice were effective in inducing the immunity.

16. Based on the accepted knowledge in the art at the time the application was made, including the Chaouat publication, one would not have expected that immunizing with *paternal* leukocytes would prove beneficial in treating an infertility condition. In fact there were a number

of studies published before the priority date of the present application which showed that immunizing human females with paternal antigen *per se* had *not* proven beneficial in treating an infertility condition, in contrast to the murine studies of Chaouat *et al.* Please refer to the attached documents:

Illeni MT, Marelli G, Parazzini F, Acaia B, Bocciolone L, Bontempelli M, Faden D, Fedele L, Maffeis A, Radici E.
Immunotherapy and recurrent abortion: a randomized clinical trial.
Hum Reprod. 1994 Jul;9(7):1247-9. (Exhibit A)

Ho HN, Gill TJ 3rd, Hsieh HJ, Jiang JJ, Lee TY, Hsieh CY.
Immunotherapy for recurrent spontaneous abortions in a Chinese population.
Am J Reprod Immunol 1991 Jan;25(1):10-5. (Exhibit B)

Cauchi MN, Lim D, Young DE, Kloss M, Pepperell RJ
Treatment of recurrent aborters by immunization with paternal cells--controlled trial.
Am J Reprod Immunol 1991 Jan;25(1):16-7. (Exhibit C)

17. This is clear evidence that at the time the applicant's application was filed, there was uncertainty as to whether "immunizing with paternal antigen" to treat an infertility condition was efficacious. In fact, there is now very compelling evidence from a NIH-funded collaborative multi-centre study which shows that immunization of women with paternal lymphoid cells stored at 4°C overnight before inoculation is still able to induce an antibody response to paternal MHC (and hence expressed paternal antigen in immunogenic form), but that these stored cells *alone* did not improve pregnancy outcome in infertility. This paper was published in 1999, ie. after the date of filing of the present application. See:

Ober C, Karrison T, Odem RR, Barnes RB, Branch DW, Stephenson MD, Baron B, Walker MA, Scott JR, Schreiber JR.
Mononuclear-cell immunisation in prevention of recurrent miscarriages: a randomised trial.
Lancet 1999 Jul 31;354(9176):365-9. (Exhibit D)

Thus even after the present filing date there was still uncertainty in the art on this issue.

18. I do not believe that the Chaouat reference provides any motivation to combine its teachings with those of Feinberg and Clark in order to arrive at the applicant's invention as claimed.

19. I consider that if the references by Feinberg *et al.*, Clark *et al.* and Chaouat *et al.* are properly interpreted in the light of the state of the art at the claimed priority date of the present application, a person of ordinary skill in the art would not have been motivated to combine the disclosures of these references in order to arrive at the invention as now claimed.

I declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by prison or fine or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of any application or patent thereon.

DECLARED at Burlington this 9th day of September 2003

David A. Clark

David A. Clark

Before me:

FULVIO DEL BIANCO
Solicitor
& Notary Public

A person empowered to witness Statutory Declarations under the laws of the Province of Ontario, Canada.